OPTIMISATION OF PROTEIN PRODUCTION IN HEK293 CELLS WITH DIFFERENT PEPTONES

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I - ABSTRACT

Mammalian cells are the natural source of many therapeutic proteins and are used as the most elaborated platform for the production of recombinant proteins with clinical applications.

For more than 15 years, *Organotechnie's Tryptone N1* has been widely documented to favor high titer protein expression post-transfection.

The HEK293 cells have been used to optimize IL-4 expression with various peptones: Vegetal Peptones versus Tryptone N1 as control.
The aim of the study is to show the efficiency of the plant origin peptones for the production of His-hIL4 construct from HEK293 cells.

The study shows that the **Peptones from Pea, Guar and Soy Proteins** enabled an improved protein production, around 150% more than our control, the Tryptone N1.
These Plant Peptones are: P112, G115 and S204.

II - INTRODUCTION

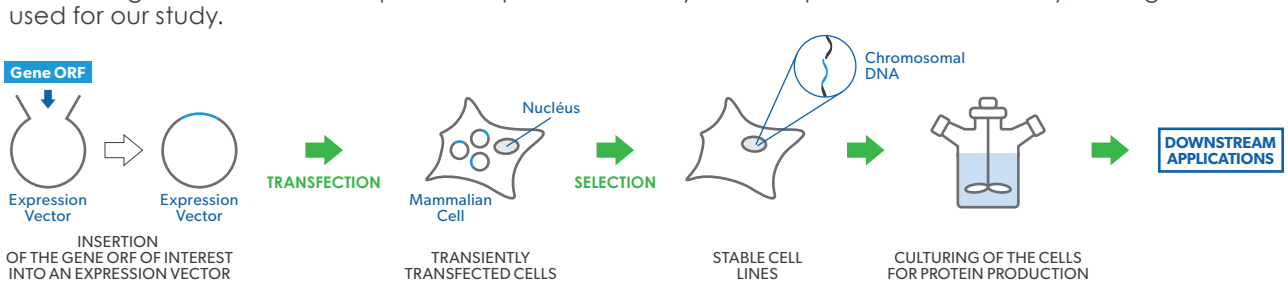
Various biological expression systems are currently used for the large-scale production of recombinant proteins. They involve microbial as well as eukaryotic host cells.
The importance of mammalian cells is increasing, as they are capable of protein folding and post-translational modifications that can express proteins with molecular structures, physical, chemical structures and biological approaching natural organism proteins.
Mammalian cells increase the possibility to obtain the same bioactivity as natural proteins.

HEK293 is the most widely used protein expression platform at the R&D stage when it comes to obtain complex therapeutic biomolecules. HEK293 cells are able to produce human proteins closest to in vivo with same glycosylation profiles, well folded and active.

Serum-free media has significant advantages in therapeutic protein biomolecules studies:
NO CONTAMINANTS FROM ANIMAL ORIGIN, REDUCED ETHICAL CONCERNS, SIMPLIFIED DSP, LOWER COST.

Serum-free media has significant advantages in therapeutic protein biomolecules studies, as follows:
no contaminants from animal origin, reduced Ethical concerns, Simplified DSP (Downstream Process), Lower cost, etc.

Supplementation of protein hydrolysates has been shown to enhance cell growth and productivity in mammalian cells.
In our study, we have worked with interleukin4 (IL4) production construct from HEK293 cells with several different vegetal origin protein hydrolysates.
Several tags can be used to improve the protein solubility and the purification efficiency, His-tag has been used for our study.



III - MATERIAL AND METHODS

Cell: HEK293-6E
Vector: pTT28
Protein: IL-4

Media composition
- FreeStyle F17 (A13835)
- GlutaMAX 1x (35050)
- Pluronic F-68 1x (24040)
- 0.5% Peptones

C-CELL Organotechnie Peptones:

Soy: S204 #17204, S203 #17203, S104 #17104, S103 #17103

Wheat: W206 #17206, W106 #17106

Pea: P112 #17112

Guar: G115 #17115

Yeast Extract: Y109 #17109

Casein: Tryptone N1 #19553

01 PRE-CULTURE
- Before transfection, pre-culture of HEK293-6E

02 TRANSFECTION MONITORING
- Transfected with pTTo/GFPq
- 0,5% Tryptone N1

03 PRODUCTION MONITORING
- Transfected with pTTo3c/SSH
- 0,5% Tryptone N1

04 PROTEIN EXPRESSION
- Transfection with pTT28 vector
- Expressed pTT28-His-IL4, 0,5% peptones
- Sample collection at T=120

05 SDS-PAGE
- Coomassie staining and Western blot anti-His tag

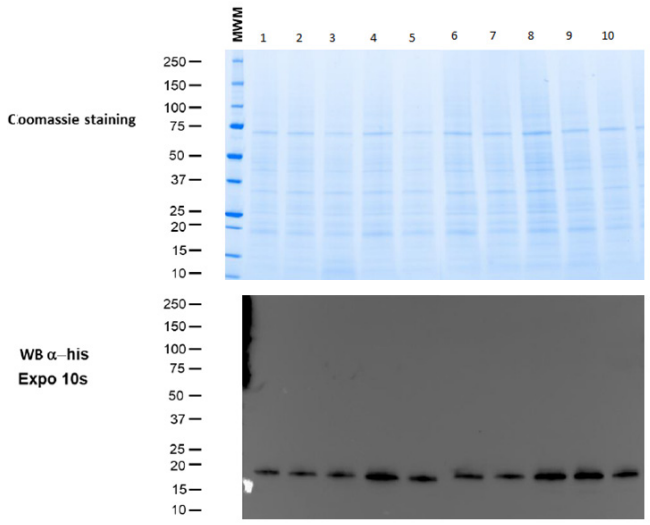
06 QUANTIFICATION
- Quantification of IL-4 in the conditioned medium from Western blot by using Image J software and Gel analysis tool

IV - RESULTS

Cell counting

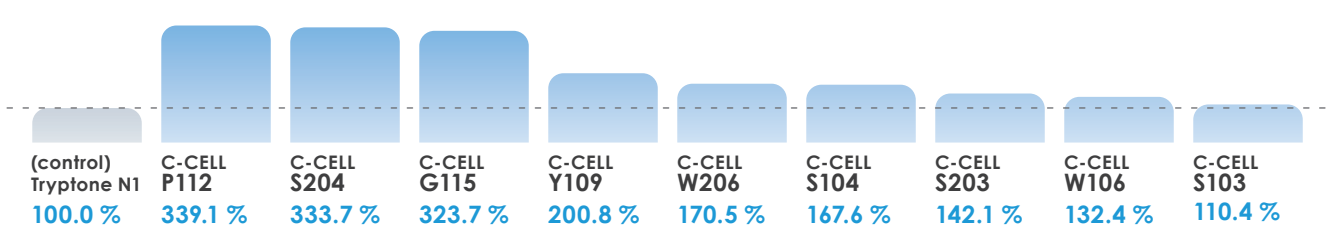
Conditions with peptones at T=120	TC20 Cell counting (cells/Ml)
hIL4 + S104	2.0 X 10E6
hIL4 + N1	1.89 X 10E6
hIL4 + S103	1.69 X 10E6
hIL4 + S204	1.63 X 10E6
hIL4 + P112	1.56 X 10E6
hIL4 + G115	1.48 X 10E6
hIL4 + Y109	1.48 X 10E6
hIL4 + W206	1.1 X 10E6
hIL4 + S203	1.02 X 10E6
hIL4 + W106	0.94 X 10E6

Verification of protein expression by SDS-page



line	peptone
1	tryptone N1
2	C-Cell S103
3	C-Cell S203
4	C-Cell S204
5	C-Cell S206
6	C-Cell S104
7	C-Cell W106
8	C-Cell P112
9	C-Cell G115
10	C-Cell Y109

Quantification of IL-4 in the conditioned medium from Western-blot



(control) Tryptone N1	C-CELL P112	C-CELL S204	C-CELL G115	C-CELL Y109	C-CELL W206	C-CELL S104	C-CELL S203	C-CELL W106	C-CELL S103
100.0 %	339.1 %	333.7 %	323.7 %	200.8 %	170.5 %	167.6 %	142.1 %	132.4 %	110.4 %

V - DISCUSSION AND CONCLUSION

An IL-4 expression study was performed to evaluate peptones' capacity to improve the yield of protein production.

Different plant peptones were compared to the Tryptone N1 which is used in the usual protocol of protein production.
There was no change in the production process, the production of IL-4 was mostly secreted in the culture medium.

The results showed that different source of vegetal peptones increased the secretion of IL-4.

The quantification of production yield from Western-blot is a semi-quantitative analysis. Therefore only the peptones showing an improved protein production, around 150% more than our control (Tryptone N1) can be considered.
Among them, the C-CELL P112, G115 and S204 showed a significant difference with high increase in protein production.